ANTIDIARRHEAL ACTIVITY OF ETHANOL EXTRACT OF LABAN BARK (*VITEX PUBESCENS* VAHL) IN MICE (*MUS MUSCULUS*).

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Abstract

Diarrhea is the second leading cause of death in children under 5 years in the world with a percentage of 16%. People especially in Kalimantan often use natural ingredients-based diarrhea treatment, one of which is laban bark, so researchers want to know the antidiarrheal activity of laban bark for diarrhea treatment. The goal is to determine the antidiarrheal effect of Laban bark extract on mice induced by *Oleum ricini* and the optimum concentration that can have an antidiarrheal effect. The methods is research design is quantitative experimental with a true experimental research design and with a pretest-posttest design with control group design. The results showed that there was a decrease in the frequency of defecation and an increase in stool consistency at doses of 250 mg/kgBW, 500 mg/kgBW, and 750 mg/kgBW. There is a difference between the parameters of the frequency of defecation and stool consistency with a significance value of <0.05. Laban bark ethanol extract Dosage of 250 mg/KgBW is better than doses of 500 mg/kgBW, and 750 mg/kgBW. The Conclusion is laban bark ethanol extract can provide antidiarrheal activity at doses of 250 mg/kgBW, 500 mg/kgBW, and 750 mg/kgBW based on the parameters of frequency of defecation and stool consistency. Doses of 250 mgkgBW have the best effect, because doses of 250 mg/kgBW and 5oo mg/kgBW are suspected to be toxic

Keywords: Antidiarrheal, Mice (*Mus musculus*), Laban skin (*Vitex pubescens* Vahl), laban bark ethanol extract can provide antidiarrheal activity

1. Introduction

According to data from the World Health Organization (WHO) and United Nation Children's (UNICEF), diarrhea is the second leading cause of death for children under 5 years old in the world with a percentage of 16%. Every year cases of diarrhea occur around 1.7 billion cases and cause about 760,000 children to die. WHO and UNICEF proposed a plan called The Integrated Global Action Plan for the Prevention and Control of Pneumonia and Diarrhea (GAPPD) which aims to reduce the number of young deaths caused by diarrhea. Diarrhea is one of the health problems in the world, including developing countries such as Indonesia (Kemenkes RI, 2018).

In 2017 it was recorded 21 times, spread over 12 provinces and 17 regencies/cities with 1725 sufferers and 34 deaths, while in 2018 it was recorded 10 times, spread over 8 provinces with 756 sufferers and 36 deaths (Kemenkes RI, 2018). Based on data from Indonesia's health profile in 2019, there were 765 post-neonatal deaths caused by diarrhea and diarrhea was the most common cause of death in children under five, namely 314 people. Diarrhea is one of the largest groups of diseases whose cases are still commonly found in South Kalimantan. Environmental factors and poor sanitation conditions are the causes of the high incidence of diarrhea. The comparison of diarrhea cases in 2008 was 54,316 cases, in 2009 there were 72,020 cases, in 2010 there were 52,908 cases, and in 2015 there were 66,765 cases (Dinas Kesehatan Provinsi Kalimantan Selatan, 2015).

The first treatment to treat diarrhea is to replace body fluids, then avoid certain foods and take probiotic and zinc supplements and take chemical-based drugs. Chemical drugs are certainly not free from side effects. Some people in Indonesia prefer to use herbal treatment rather than chemical drugs, especially for diarrheal diseases. Medicinal plants used as antidiarrheals have an astringent or chelating effect that can shrink the intestinal mucous membrane thereby reducing fluid expenditure during diarrhea, besides that it also has an anti-inflammatory and antibacterial effect.

One type of plant that can be used for the treatment of diarrheal diseases is laban bark (*Vitex pubescens* V). In the area of Central Kalimantan, especially in the city of Sampit, the Dayak community in Lape Village use the bark of laban (*Vitex pubescens* Vahl) as a treatment for diarrhea and drink it as a drink instead of tea, old bark and young bark are the parts used in this plant. In addition, the bark of Laban is efficacious as a medicine for stomachaches, diarrhea, colds and body fresheners. Laban bark (*Vitex pubescens* Vahl) contains alkaloids, flavonoids, tannins, saponins and terpenoids. According to research by Rahma 2020, alkaloids, flavonoids and saponins have antibacterial activity by showing antibacterial inhibition with various different mechanisms of action (Adelina et al., 2014; Hermansah et al., 2015; Rahma, 2020). Several studies have shown that flavonoids have antidiarrheal activity by inhibiting intestinal motility, thereby reducing fluid and electrolyte secretion. Flavonoids inhibit the release of acetylcholine in the gastrointestinal tract. Inhibition of acetylcholine release leads to reduced activation of acetylcholine receptors that regulate gastrointestinal motility and smooth muscle contraction. This compound is thought to play a role in influencing the decrease in the frequency of diarrhea. Tannins can reduce the intensity of diarrhea because it acts as an astringent where this substance shrinks the intestinal mucous membrane and shrinks the pores so that it will inhibit the secretion of fluids and electrolytes so that it will affect the frequency of diarrhea. In addition, the astringent properties of tannins will make the small intestine more resistant to chemical stimuli that cause diarrhea, such as from bacterial toxins and the induction of diarrhea by castor oil (Toemon et al., 2019).

Several previous research results reported that laban wood extract as an antibacterial against *E.coli* bacteria that causes diarrhea was carried out in vitro (Rahma, 2020). Based on the above, a study was conducted to test the antidiarrheal activity of the ethanol extract of the bark of Laban Kayu in vivo in mice (*Mus musculus*).

Problem Formulation

Based on the background that has been explained, a problem can be formulated as follows:

1. Does Laban V.pubescens bark extract have antidiarrheal activity against mice induced by *Oleum ricini*?
2. What is the optimum concentration of *Laban* V.*pubescens* bark extract which can have an antidiarrheal effect?

Research Objectives

This research aims as follows:

1. To determine the antidiarrheal effect of V.*pubescens laban* bark extract on mice induced by *Oleum ricini*.
2. Knowing the optimum concentration of V.*pubescens laban* bark extract which can have an antidiarrheal effect.

Research Benefits

This research is expected to provide the following benefits:

1. For researchers
   This study was able to determine that the ethanol extract of the bark of Kayu Laban (*Vitex pubescens* V) has antidiarrheal activity against mice.
2. For education

This study is expected to provide motivation to other researchers as well as further researchers to conduct further research on the antidiarrheal activity of the bark of Laban Wood (*Vitex pubescens* V) on mice.

3. For the community

   This research can provide scientific data that can support the use and development of the bark of Laban Wood (*Vitex pubescens* V) as a traditional medicine that has an antidiarrheal effect and as an alternative to diarrhea medicine.

2. Materials and Methods

Materials

The materials used were laban bark, loperamide HCl, new diatabs, aquadest, 0.5% Na CMC 0.5%, *Oleum ricini*, mice (*Mus musculus*), aquadest, lieberman burchard reagent, mayer reagent, dragendorff reagent. , HCl, magnesium powder, gelatin, $FeCl_3$

Methods

This type of research is an experimental quantitative research with a true experimental research design, with a pretest-posttest with control group design that is carried out by two groups, one group is given the intervention and the other group as a control which will be observed before and after the intervention (Suharman et al., 2016).

Population and Research Sample

1. Population

   The population is all the elements that are the object of research (Masturoh & Anggita T, 2018). In this study, the population used was mice (*Mus musculus*).

2. Sample

   The sample is part of the total population studied and conclusions drawn (Masturoh & Anggita T, 2018). The samples used in this study were mice aged 2-3 months with a weight of 20-30 grams. Mice were divided into 6 groups where 1 group consisted of 5 mice. The group consisted of negative control with 0.5% Na CMC administration (Sugipratiwi *et al*., 2016). Positive control with loperamide HCl and New diatab, treatment group with various doses of laban bark ethanol extract 250 mg, 500 mg, 750 mg (Rahma, 2020).

   Inclusion Criteria

   - Male White Mice (*Mus musculus*)
   - Gender Male 2-3 months old
   - Weight 20-30 grams
   - Healthy Mice

   Exclusion Criteria

   - Unhealthy mice
   - Appearance of hair loss
   - Less active
   - Exudate from the nose
   - Rash on the skin
   - Died during research

Work procedures

1. Simplicia making

   First of all, the simplicia is prepared. The old laban bark was obtained from Sampit City, Central Kalimantan. The second stage is sorting to separate from impurities such as sand, gravel, stone etc. The third stage is washing using clean running water. The purpose of washing is to further clean up the remnants of foreign organic matter that is still attached during wet sorting. The fourth stage is chopping by slicing/cutting the laban bark thinly using a knife or a special chopper machine. The purpose of the extension is to facilitate the drying process. The fifth stage is drying by drying in direct sunlight. The sixth stage is dry sorting to separate from foreign objects left behind during drying. The last stage in the preparation of simplicia is packing and packaging. Simplicia is stored in a hygroscopic and airtight glass container to prevent it from rotting and overgrown with microbes.

2. Preparation of Laban Bark Extract (*Vitex pubescens* Vahl)
   The dried laban bark simplicia was put into a maceration vessel then added 96% ethanol solvent then stirred and allowed to stand for 3 x 24 hours. Furthermore, stored in a tightly closed container and protected from sunlight. The extraction results are then filtered, the filtrate is then concentrated with a water bath until a thick extract is obtained and then calculate the yield to determine the percentage of the resulting extract (Hasnaeni *et al.*, 2019; Lina & Astutik, 2020; Rizal *et al*., 2016).

$$\% \text{ Yield} = \frac{\text{final extract weight}}{\text{initial simplicia weight}} \times 100\%$$

3. Phytochemical Screening
   The purpose of phytochemical screening is to determine secondary metabolites contained in laban bark (*Vites pubescens* V). Secondary metabolites that are considered as antidiarrheal are alkaloids, flavonoids, tannins, saponins and terpenoids (Anggraeni *et al*., 2020; Hasnaeni *et al*., 2019)
   1) Alkaloids
      total of 0.5 grams of the extract was dissolved in 10 ml of 96% ethanol. The test using Dragendorff's reagent will show an orange color close to red if it is positive for alkaloids. The test using Mayer's reagent will form a white precipitate if it is positive for alkaloids (Rahma, 2020)
   2) Flavonoids
      Using the shinode test, a sample of 0.5 grams of simplicia was used, then dissolved in 10 ml of ethanol, added 0.1 grams of magnesium powder (Mg) and 5-10 drops of 5M hydrochloric acid (HCl) and observed. A change in red, yellow, orange color indicates a positive presence of flavonones, flavonols and dihydroxyflavonols (Hasibuan *et al.*, 2020)
   3) Tannins
      Using the 10% gelatin test by adding the extract with 1 ml of 10% gelatin. If it is positive for tannin, it will form a white precipitate. Using the FeCl3 test, a sample of 0.5 grams was taken dissolved with 10 ml of distilled water and then 2 drops of 1% FeCl3 reagent were added. If a blue or blackish green color is formed, it indicates the presence of tannins (Hasibuan *et al.*, 2020)
   4) Saponins
      Using the foam test, 0.5 grams of simplicia powder was taken, added 10 ml of heated distilled water, then shaken vigorously for 10 seconds. Positive results contain saponins if foam is formed as high as 1 to 10 cm which lasts for not less than 10 minutes and when 1 drop of 2N hydrochloric acid is added, if the foam does not disappear (Hasibuan *et al.*, 2020).
   5) Terpenoids
      Using the Liebermann Burchard test (anhydrous acid and concentrated sulfuric acid). The extract was dissolved in 20 ml of 96% ethanol. A positive reaction indicates the formation of a bluish-green (Hasibuan *et al.*, 2020).

3. Preparation of 0.5% Na CMC solution
   Weighed 0.5 grams of Na CMC, then dissolved with 100 ml of hot water, after that it was put in a 50 ml volumetric flask, then made up with distilled water to 100 ml.

4. Preparation of loperamide HCl solution
   The loperamide HCl suspension was made by grinding in a mortar 1 tablet (dose of 2 mg/70kgBB), then loperamide HCl powder was dissolved in 10 ml of 0.5% Na CMC colloidal solution and then crushed ad homogeneously.
   Conversion of dose to mice = 2 mg × 0.0026 = 0.0052/20grBB mice
5. Making new diatabs solution
   The new diatabs suspension was made by grinding in a mortar 2 tablets (1,200 mg/70kgBB), then the new diatabs powder was dissolved in 10 ml of the solution.
   Conversion of dose to mice = 1.200× 0.0026 = 3.12/20grBB mice
6. Preparation of stock solution of laban bark ethanol extract
   A stock solution of laban bark ethanol extract was made at a dose of 250 mg/kgBW, 500 mg/kgBW, 750 mg/kgBW. Weigh the ethanol extract of the laban bark and then dissolve it into 10 ml of 0.5% Na CMC colloid solution and grind it homogeneously. The dose conversion factor for humans (70 kg) in mice weighing 20 g is 0.0026 (Rahma, 2020; Rizal *et al.*, 2016)
   Conversion dose 250 mg = (250 mg)/(1000 g) × 20 g = 5 mg/20 gBW mice
   Conversion dose 500 mg = (500 mg)/(1000 g) × 20 g = 10 mg/20 gBW mice
   Conversion dose 750 mg = (750 mg)/(1000 g) × 20 g = 15 mg/20 gBW mice
7. Preparation of test animals
   The mice were acclimatized for 7 days in order for the mice to adapt to the new environment. Before starting the experiment, mice were fasted for about 1 hour only given drinking water. It aims to empty the intestinal contents from the influence of the food consumed by mice, thus facilitating the absorption process in the gastrointestinal tract when induced with *Oleum ricini*. This test uses *Oleum ricini* as a diarrhea inducer (Anggraeni *et al*., 2020)
8. Antidiarrheal activity test
   The first test was done by looking at the activity of diarrhea in mice for 4 hours. Mice in each group were separated in a container. This antidiarrheal test uses a protection method. In this method, the mice were given oral treatment according to the group that had been determined (Suliska *et al.*, 2019) namely:

Table 1: Treatment Groups Antidiarrheal Activity Test Ethanol Extract of Laban Bark (Vitex pubescens Vahl)

Name of Group		Treatment
Group 1	Negative control group	Suspensi Na CMC 0,5%
Group 2	Positive control group A	Loperamide HCl
Group 3	Positive control group B	New diatabs
Group 4	Dosage group 1	Ekstrak etanol kulit kayu laban dosis 250 mg/kgbb
Group 5	Dosage group 2	Ekstrak etanol kulit kayu laban dosis 500 mg/kgbb
Group 6	Dosage group 3	Ekstrak etanol kulit kayu laban dosis 750 mg/kgbb

Thirty minutes mice were induced with *Oleum ricini* orally as much as 0.5ml/20gBW. After that do observations for 4 hours with an interval of every 15 minutes. After that, observe diarrhea with parameters, namely stool consistency and frequency of defecation (Anggraeni *et al*., 2020; Lina & Astutik, 2020; Rizal *et al*., 2016).

3. Results and Discussion

Research entitled Antidiarrheal Activity Test of Ethanol Extract of Laban Bark (*Vitex pubescens* Vahl) in Mice (*Mus musculus*) obtained the following results:

1. Determination Results

   In this study using a laban tree (*Vitex pubescens* Vahl) whose bark was taken, to give the truth of the laban bark being studied, a determination was made at the Research Center for Plant Conservation and Botanical Gardens - LIPI. The results of the determination of the laban tree plant (*Vitex pubescens* Vahl) stated that it was true that the plant studied was of the type *Vitex pinnata* L. (synonym *Vitex pubescens* Vahl), Lamiaceae, laban.

2. Making Simplicia

   The simplicia was made at the researcher's house. The manufacture of simplicia starting from chopping to packing was carried out on December 24, 2020. The weight of simplicia obtained was 1,914 kg from 1 sack of laban bark which was directly taken from the forest in the Pelangsian area, Sampit City, Central Kalimantan Province, Indonesia.

3. Extraction of Laban Bark (*Vitex pubescens* Vahl)

The results obtained from the extraction of Laban Bark (*Vitex pubescens* Vahl) are as follows:

Table 2: Laban Bark Extract Results (Vitex pubescens Vahl)

Laban Bark Extract (*Vitex pubescens* Vahl)	Result
The weight of the simplicia sample used	950 gram
Amount of ethanol 96&	13 liter
Liquid extract	12 liter
Thick extract	62,39 gram
% Yield	6,57%

from phytochemical screening, the metabolites contained in laban bark (Vitex pubescens Vahl) are alkaloids, flavonoids, tannins and saponins.

Table 3: Phytochemical Screening Ethanol Extract of Laban Bark (Vitex pubescens Vahl)

secondary metabolites	**Reagens**	**Result**	**Description**	**+/-**
Alkaloids	Dragendorf		orange close to red	+
	Mayer		white precipitate	+
Flavonoids	Shinoda		red	+

Tannins	Gelatin 10%		white precipitate	+
	FeCl 1%		green close to black	+
Saponin	Aquadest + HCl 2N		1.5 cm froth lasts more than 10 minutes and does not disappear when 2N . HCl is added	+
Terpenoid	Lieberman Burchard		Brown liquid	-

Information:

(+) = contains secondary metabolites

(-) = no secondary metabolites

The following are the results of the antidiarrheal activity test

Table 4: Differences in Pre-test – Post-test Statistical Data Analysis

	Na CMC 0,5%	Loperamide HCl	New Diatabs	EEKKL 250 mg	EEKKL 500 mg	EEKKL 750 mg
Consistency	.002*	.046	.241	.319	.058	.182
Frequency	.008*	.030	1.000	.319	.401	.519

Description : EEKKL = Laban Bark Ethanol Extract

* = Significantly different between the pre test and post test groups

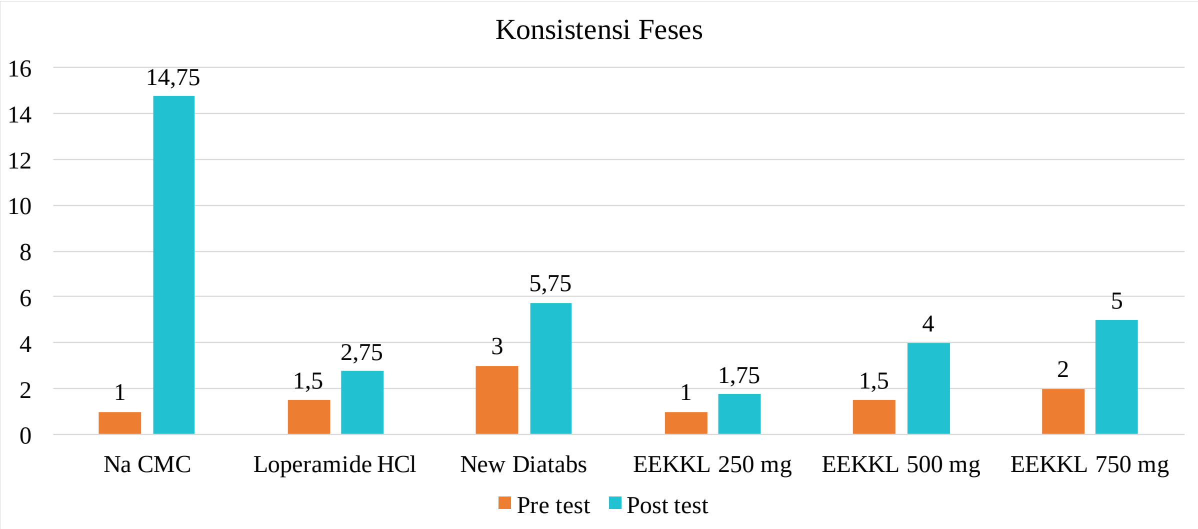

Figure 1: Graph of Differences in Diarrhea Consistency between Pre test and Post Test

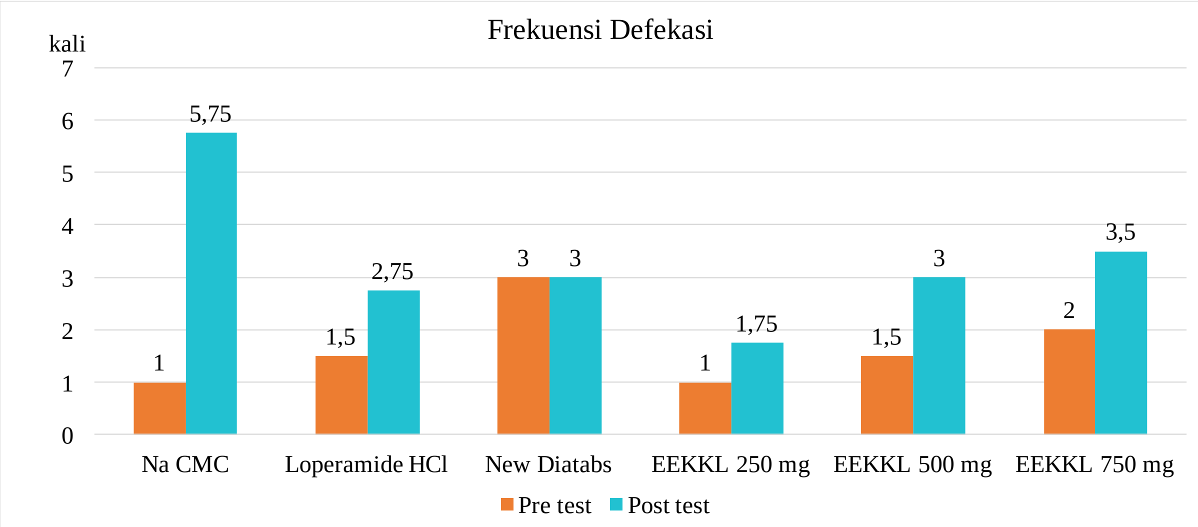

Figure 2: Graph of the Difference in Diarrhea Frequency between Pre test and Post Test

Table 5: Significance Results of Analysis Parameters of stool consistency and frequency of diarrhea in positive and negative controls

	Na CMC 0,5%	**Loperamide HCl**	**New Diatabs**	**EEKKL 250 mg**	**EEKKL 500 mg**	**EEKKL 750 mg**
CONSISTENCY						
1. Na CMC 0,5%	-	.021*	.021*	.020*	.020*	.021*
2. Loperamide HCl	.021*	-	.663	.549	.767	.559
3. New Diatabs	.021*	.663	-	.306	.661	.885
4. EEKKL 250 mg	.020*	.549	.306	-	.219	.240
5. EEKKL 500 mg	.020*	.767	.661	.219	-	.884
6. EEKKL 750 mg	.021*	.559	.885	.240	.884	-

FREQUENCY						
1. Na CMC 0,5%	-	.028*	.019*	.019*	.036*	.027*
2. Loperamide HCl	.028*	-	.237	.549	.544	.765
3. New Diatabs	.019*	.237	-	.356	.063	.559
4. EEKKL 250 mg	.019*	.549	.356	-	.215	.881
5. EEKKL 500 mg	.036*	.544	.063	.215	-	.369
6. EEKKL 750 mg	.027*	.765	.559	.881	.369	-

Description : EEKKL = Laban Bark Ethanol Extract
• = Significantly different

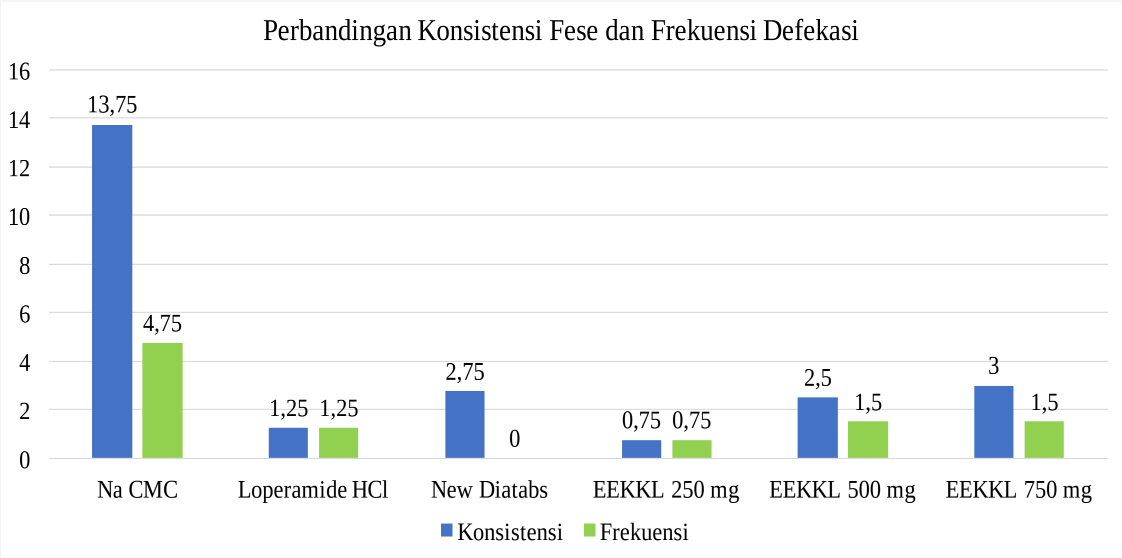

Figure 3: Graph of Differences in Consistency and Frequency of Diarrhea

Based on the analysis of the Kruskall Wallis test data in appendix 1 on the parameters of the frequency of defecation and stool consistency, it has a significant value <0.05, which means that there is a significant difference between each treatment group. Based on the results of the t test data analysis on the parameters of the frequency of defecation and stool consistency in the loperamide HCl group, new diatabs and all groups, the dose variation was not significantly different between the pre test and post test, while the Na CMC group was significantly different. This means that the conditions are the same between before being treated and before being treated from the parameters of the frequency of defecation and stool consistency. The pre-test and post-test values of stool consistency were obtained from the basic average of each group, while the pre-test and post-test values of the frequency of defecation were obtained from the average number of defecation frequencies from each group. Based on the results of the analysis of table 4 Mann Whitney test on the parameters of frequency of defecation and stool consistency between the loperamide HCl, new diatabs, EEKKL 250 mg, EEKKL 500 mg and EEKKL 750 mg groups had significant differences with the Na CMC group, while all dose groups were not different with loperamide HCl and new diatabs groups, which means that the third dose of EEKKL has the same activity as loperamide HCl and new diatabs.

According to Wells et al., 2016 diarrhea is the occurrence of an increase in the frequency of bowel movements and a decrease in stool consistency compared to normal bowel conditions in an individual.

This is in accordance with the results of the study which can be seen in Figure 3 The difference in consistency and frequency parameters by comparing the positive control can be seen that all dose variations, namely the dose of EEKKL 250 mg, EEKKL 500 mg and EEKKL 750 mg, have been shown to have antidiarrheal activity. The dose of EEKKL 250 mg has the best effect, because the dose of EEKKL 500 mg and EEKKL 750 is suspected to be toxic if seen from the graphic data in Figure 3 According to research on acute toxicity tests in mice, the dominant toxic effect is parasympathomimetic or parasympathetic nerve stimulation, causing stimulation of the digestive tract and intestinal peristalsis which eventually causes diarrhea and even bloody diarrhea and increased urine production and the number of animals that die in each dose (Jumain, Syahruni, 2018; Rasyid *et al.,* 2012*).*

The decrease in the frequency of defecation and the increase in the consistency to become solid in the administration of ethanolic extract of laban bark is caused by secondary metabolites, namely tannins which are astringent which are able to shrink the intestinal mucous membrane and shrink the pores, thereby inhibiting the secretion of fluids and electrolytes, making feces solid. Tannins can reduce the intensity of diarrhea and the frequency of defecation. In addition, the astringent properties of tannins will make the small intestine more resistant to chemical stimuli that cause diarrhea, such as from bacterial toxins and the induction of diarrhea by castor oil (Toemon et al., 2019).

The presence of antidiarrheal activity in the ethanolic extract of Laban bark is also because it contains secondary metabolites of flavonoids. The mechanism of flavonoids in stopping diarrhea induced by *Oleum ricini* is by inhibiting intestinal motility thereby reducing fluid and electrolyte secretion, this is the same as loperamide HCl which can also inhibit intestinal motility.

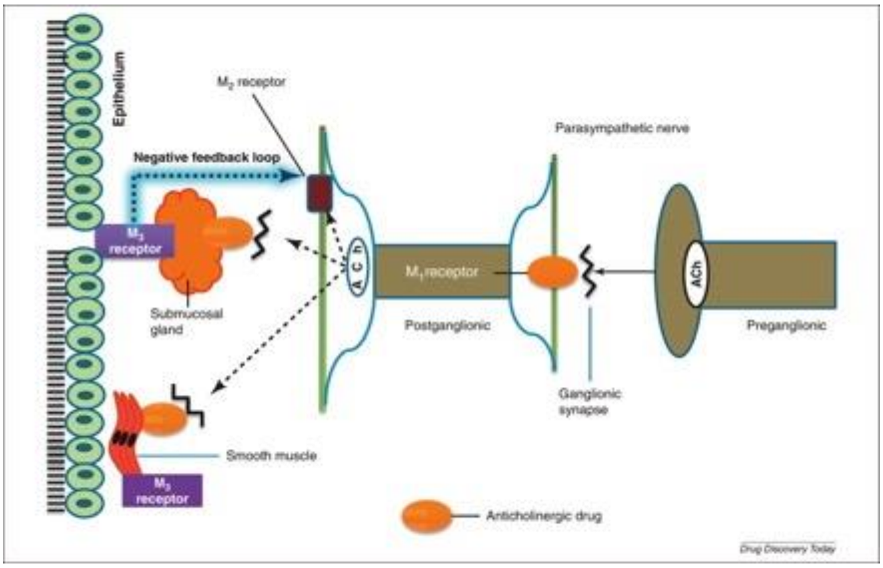

Figure 4: The mechanism of flavonoids in inhibiting the release of acetylcholine (Prakash *et al*., 2013)

Flavonoids inhibit the release of acetylcholine in the gastrointestinal tract. Inhibition of acetylcholine release is a competitive antagonist by blocking the binding of acetylcholine to cholinergic receptors, namely muscarinics found in smooth muscle, resulting in reduced activation of acetylcholine receptors that regulate gastrointestinal motility and smooth muscle contraction. This compound is thought to play a role in influencing the decrease in the frequency of defecation (Toemon *et al*., 2019)

Another secondary metabolite compound that plays a role in antidiarrheal activity is the content of alkaloids and saponins. This compound has antibacterial activity (Anggraito *et al,* 2018 Rahma, 2020).

4. Conclusion

Based on the results of the Antidiarrheal Activity Test of Laban Bark Ethanol Extract (*Vitex pubescens* Vahl) in Mice (*Mus musculus*) it can be concluded that the ethanolic extract of Laban bark contains secondary metabolites in the form of alkaloids, flavonoids, tannins, saponins, and ethanolic extract of Laban bark. can provide antidiarrheal activity similar to that of loperamide HCl at doses of 250 mg/kgBW, 500 mg/kgBW, and 750 mg/kgBW against white mice induced by *Oleum ricini* seen based on the parameters of frequency of defecation and stool consistency. Laban bark ethanol extract at a dose of 250 mg/KgBW had the best effect because the dose of 500 mg/kgBW and 750 mg/kgBW were suspected to be toxic.

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Appendix

Appendix 1. Data Analysis With Kruskall Wallis Test

Ranks

	Perlakuan	N	Mean Rank
Consistency	Na CMC 0,5%	4	22.50
	Loperamide HCl	4	13.00
	New Diatabs	4	12.13
	EEKKL 250 mg	4	6.75
	EEKKL 500 mg	4	10.75
	EEKKL 750 mg	4	9.88
	Total	24	
Frequency	Na CMC 0,5%	4	21.75
	Loperamide HCl	4	16.25
	New Diatabs	4	6.88
	EEKKL 250 mg	4	9.75
	EEKKL 500 mg	4	14.25
	EEKKL 750 mg	4	6.13
	Total	24	

Test Statistics[a,b]

	Consistency	frequency
Chi-Square	11.727	15.108
df	5	5
Asymp. Sig.	.039	.010

a. Kruskal Wallis Test

b. Grouping Variable: treatment